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(54) Title: VAGINAL ACTIVE AGENT DELIVERY PROCEDURES AND FORMULATIONS THEREOF

(57) Abstract

Intra–vaginal delivery procedures including compositions and units therefor whereby an effective releasable amount of oestradiol 17$\beta$ is released to achieve an efficacious effect insofar as oestrus expression is concerned. Preferably a cyclodextrin is utilised to enhance absorption.

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# VAGINAL ACTIVE AGENT DELIVERY PROCEDURES AND FORMULATIONS THEREOF

## TECHNICAL BACKGROUND

The present invention relates to intra-vaginal delivery or dosage units, compositions suitable therefor, the use thereof and related means and methods.

In our New Zealand patent specification numbers 207341, 286492 (PCT/NZ97/00052) and 314572/314175 (PCT/NZ98/00011) there are disclosed procedures applicable in a range of different animals insofar as the synchronisation of the onset of oestrus is concerned.

In our EAZI-BREED™, CIDR™ "Controlled Breeding and Reproductive Management" publication there is disclosed the use of an oestradiol co-treatment (ie; CIDIROL™) with the use of our progesterone releasing CIDR™ intra-vaginal inserts in cattle for treatment of anoestrus or for oestrus synchrony. Current practice when selecting an oestradiol co-treatment, for use with a progesterone releasing intra-vaginal insert (such as our CIDR-B™ device), is to select oestradiol benzoate by intramuscular (i.m.) injection (eg; CIDIROL™).

In the past attempts at vaginal administration of oestradiol benzoate by using a capsule has resulted in poor oestrus expression and poor fertility. This outcome has been notwithstanding doses typically 10 times higher than those usually administered by intra-muscular injection.

## BACKGROUND ART

Our invention is directed towards a means whereby delivery of an active agent such as that to serve the role of previously used oestradiol benzoate is improved using and/or despite using a vaginal administration procedure.

It is therefore an object of the present invention to provide intra-vaginal delivery systems, dosage units, compositions, methods of use thereof and related means and methods which will be useful (preferably in conjunction with a progesterone releasing intra-vaginal insert such as our CIDR-B™ range of intra-vaginal inserts) in animals.

We have determined in cattle that the lack of performance when using the

vaginal administration of oestradiol benzoate is not attributed to the dose, as typically doses are 10 times higher than those administered by intra-muscular injection, but to poor and variable absorption of oestradiol benzoate following vaginal administration. Typically peak plasma oestradiol concentrations following vaginal administration of 10 mg oestradiol benzoate range from 2 to 5 pg/ml. The peak in plasma levels is obtained between 2 and 48 hours following administration. In comparison peak plasma oestradiol concentrations following i.m. injection of 1 mg oestradiol benzoate range from 8 to 13 pg/ml. The peak in plasma level is obtained by approximately 2 hours following administration and maintained for up to 24 hours following treatment.

We believe similar effects occur in other animal species, viz, buffalo, pig, goat, sheep and deer.

As a result of our research in cattle we have determined that the natural oestradiol 17β rather than the synthetic analogue oestradiol benzoate can be effectively administered vaginally despite the fact that if given intra-muscularly oestradiol 17β was known to be shorter acting than the intra-muscularly efficacious oestradiol benzoate analogue thereof.

Our research in cattle has established that irrespective of whether or not that it is oestradiol 17β or an analogue thereof (such as oestradiol benzoate) that is delivered intra-vaginally an efficacious delivery thereof is possible in conjunction with an appropriate agent.

Our research has also established in cattle that intra-vaginal delivery of oestradiol 17β in preference to its analogues or the delivery of oestradiol 17β and/or its analogues in conjunction with at least one cyclodextrin can maintain serum levels of the active metabolite above normal for at least 24 hours.

We believe each, any or all of these findings are also appropriate for other animal species, viz, buffalo, pig, goat, sheep and deer, where such a regime might from time to time be warranted.

**DISCLOSURE OF THE INVENTION**

Accordingly in a first aspect the present invention consists in **an intra-vaginal delivery unit or composition** having an effective releasable amount of

oestradiol 17β to achieve an efficacious effect insofar as oestrus expression is concerned.

In a further aspect the present invention consists in **an intra-vaginal delivery unit or composition** having an effective releasable amount of oestradiol 17β to achieve an efficacious effect insofar as oestrus expression is concerned, and having, in addition, an agent able to enhance the absorption of the oestradiol 17β or on analogue via the vaginal route and/or the maintenance thereof in the blood of the recipient host.

In still a further aspect the present invention consists in **an intra-vaginal delivery unit or composition** having an effective amount of an active material or pro-drug thereof to achieve oestrus synchronisation and an effective amount of an agent to enhance the absorption by the vaginal route of the active material or pro-drug and/or the maintenance of the active material, the pro-drug or the metabolites in the blood of the recipient host.

Preferably the active material is a steroid and preferably said active material is oestradiol 17β or some synthetic analogue thereof, eg oestradiol benzoate.

Preferably said agent to enhance absorption is selected from the cyclodextrin family and preferably is hydroxypropyl β-cyclodextrin or γ-cyclodextrin.

In still a further aspect the present invention consists in **a pharmaceutical formulation to synchronize oestrus,** which comprises or includes,

(a) an active material or pro-drug to achieve oestrus synchronization;

(b) an agent (i) to enhance the absorption of the active material or pro-drug and/or (ii) to ensure the appearance of the active material or active metabolite in the plasma in concentrations above those observed prior to treatment for at least 24 hours following treatment.

Preferably the formulation is intended for administration by placement of a capsule within the vaginal of an animal.

Preferably the formulation is a capsule, tablet or similar product.

Preferably the active material or pro-drug is a steroid such as oestradiol benzoate or oestradiol 17β.

Preferably the agent to enhance absorption of an active material or pro-drug

or to provide the longer blood serum presence of the active metabolite is a cyclodextrin.

Preferably the cyclodextrin is one or more of β-cyclodextrin, γ-cyclodextrin and hydroxypropyl β-cyclodextrin.

Preferably the ratio of agent (eg; cyclodextrin) to enhance absorption to active material or pro-drug is less than to 3:2 (agent:active) by molecular amount, that is 3 moles of cyclodextrin to every 2 moles of active.

Preferably the intra-vaginal dosage unit has from 1.2 to 7.2 mg of oestradiol 17 [or an analogue equivalent amount, eg; from 10 to 30 mg if analogue is oestradiol benzoate] and from 6 to 150 mg cyclodextrin(s),

and optionally other excipients, solid or liquid,

and, preferably, if a capsule, is encased in a material such as gelatin that will release the capsule contents into vaginal fluids.

In still a further aspect the present invention consists in **a pharmaceutical formulation** containing an active material or pro-drug for the treatment of oestrus synchronization which elevates plasma oestradiol concentrations and/or the active metabolite(s) thereof for at least 24 hours.

In still a further aspect the present invention consists in **the use of an intra-vaginal dosage unit or composition or any other formulation** in accordance with the present invention substantially as hereinbefore described.

In yet a further aspect the present invention consists in **a method of providing an elevated concentration of an oestrus affecting or and/or oestrus expression affecting steroid or pro-drug thereof** (such as oestradiol 17β or any synthetic analogue thereof, eg oestradiol benzoate) or **efficacious metabolites thereof** which comprises intra-vaginally administering (preferably in a dosage unit) either

(i) an effective amount of oestradiol 17β or

(ii) an effective amount of the steroid (or analogue(s)) or pro-drug(s) thereof in conjunction (and preferably and effective amount) with an effective agent to enhance the absorption by the animal of the steroid, prodrug or metabolites thereof via its vagina and/or

to achieve an appearance of the active material or active metabolite in the plasma of the animal in concentrations above those observed prior to treatment for at least 24 hours following treatment.

Preferably said formula dosage unit is in the form of a capsule, tablet or similar product and may, for example, be associated with a delayed release mechanism of some intra-vaginal device adapted to release some preceding medicament.

A suitable source of cyclodextrins are the products BETA W7 HP hydroxypropyl β-cyclodextrin, BETA W7 β-cyclodextrin and GAMMA W8 γ-cyclodextrin from Wacker Chemicals Australia, Victoria, Australia.

A suitable source of oestradiol benzoate is from ICN Biomedical, Ohio, USA.

A suitable source of oestradiol 17β is from Sigma Chemical Company, USA.

The invention consists in the foregoing and also envisages constructions of which the following gives examples.

## DETAILED DESCRIPTION OF THE INVENTION

Preferred forms of the present invention will now be described with reference to the accompanying drawings in which:

**Figure 1** is a plasma oestradiol concentrations following intramuscular injection of 0.72 mg (closed square) or vaginal administration of 7.2 mg (open square) of oestradiol 17β. Error bars are standard error means (n=3).

**Figure 2** is a plasma oestradiol concentrations following vaginal administration of 10 mg oestradiol benzoate (open square), 10 mg oestradiol benzoate with 1:1 molar ratio β-cyclodextrin (open diamond), 10 mg oestradiol benzoate with 1:1 molar ratio hydroxypropyl β-cyclodextrin (open circle) or 10 mg oestradiol benzoate with 1:1 molar ratio γ-cyclodextrin (open triangle). Error bars are standard error means (n=4).

**Figure 3** is a plasma oestradiol concentrations following vaginal administration of oestradiol 17β 7.2 mg (open square), 7.2 mg with 1:1 molar ratio β-cyclodextrin (open diamond), 7.2 mg with 1:1 molar ratio hydroxypropyl β-cyclodextrin (open circle) or 7.2 mg with 1:1 molar ratio γ-cyclodextrin (open triangle). Error bars are standard error means (n=4).

**Figure 4** is a plasma oestradiol concentration following vaginal administration of 1.2 mg (closed diamond), 2.5 mg (closed square) or 7.2 mg (closed triangle) oestradiol 17β with 0.5:1 molar ratio of γ-cyclodextrin to oestradiol 17β. Error bars are standard error means (n=4).

**Figure 5** is a plasma oestradiol concentration following vaginal administration of 1.2 mg (closed diamond), 2.5 mg (closed square) or 7.2 mg (closed triangle) oestradiol 17β with 1:1 molar ratio of γ-cyclodextrin to oestradiol 17β. Error bars are standard error means (n=4).

**Figure 6** is a plasma oestradiol concentration following vaginal administration of 1.2 mg (closed diamond), 2.5 mg (closed square) or 7.2 mg (closed triangle) oestradiol 17β with 3:2 molar ratio of γ-cyclodextrin to oestradiol 17β. Error bars are standard error means (n=4).

**Figure 7** is a area under the plasma oestradiol concentration against time curve (AUC) following vaginal administration of 1.2 mg, 2.5 mg or 7.2 mg oestradiol 17β with γ-cyclodextrin to oestradiol 17β molar ratio of 0.5 (closed diamond), 1 (closed square) or 1.5 (closed triangle). Error bars are standard error means (n=4).

**Figure 8** is a time to maximum plasma concentration (tmax) following vaginal administration of 1.2 mg, 2.5 mg or 7.2 mg oestradiol 17β with γ-cyclodextrin to oestradiol 17β molar ratio of 0.5 (closed diamond), 1 (closed square) or 1.5 (closed triangle). Error bars are standard error means (n=4).

**Figure 9** is a maximum plasma oestradiol concentration (Cmax) following vaginal administration of 1.2 mg, 2.5 mg or 7.2 mg oestradiol 17β with γ-cyclodextrin to oestradiol 17β molar ratio of 0.5 (closed diamond), 1 (closed square) or 1.5 (closed triangle). Error bars are standard error means (n=4).

**Figure 10** is a plasma oestradiol concentration at time=0 and time=24 hours post vaginal administration of various doses of oestradiol 17β (1.2, 2.5 or 7.2 mg) with various rations of γ-cyclodextrin (0.5, 1, 1.5 molar ration of γ-cyclodextrin to oestradiol 17β). Error bars are standard error means (n=4). * Denotes a significant difference between the plasma oestradiol concentration at time=0 and time=24 hours ($p<0.050$).

The use of oestradiol 17β and not the synthetic analogue oestradiol benzoate has not been firmly established due to the poor results with vaginally administered oestradiol benzoate and a perception that the shorter acting oestradiol 17β would not be as effications as the longer acting oestradiol benzoate. When oestradiol 17β in a dose equivalent to 1 mg oestradiol benzoate, i.e. 0.72 mg, is administered by i.m. injection in cattle the plasma oestradiol concentration rapidly rises to a maximum of approximately 100 pg/ml, followed by a rapid decline to pre injection levels by 24 hours following injection. See Figure 1. Because of this rapid decline in plasma oestradiol levels an oestradiol 17β dose of 5 mg is commonly used to ensure adequate plasma oestradiol concentrations to achieve the same effect as 1 mg of oestradiol benzoate.

We have found that unlike oestradiol benzoate vaginally administration oestradiol 17β is well absorbed, with a dose of 7.2 mg achieving a peak plasma concentration of between 10 and 20 pg/ml within four hours following administration, and the plasma oestradiol levels are elevated for at least 24 hours when oestradiol 17β is vaginally administered compared to the more rapidly cleared i.m. injection of oestradiol 17 .

We have found that the cyclodextrins improve the vaginal absorption of oestradiol benzoate. We have found that β or γ-cyclodextrin approximately double the plasma oestradiol concentration when vaginally administered with oestradiol benzoate (10 mg) compared with oestradiol benzoate administered without cyclodextrin. See Figure 2.

Furthermore we have found that the cyclodextrin hydroxypropyl β-cyclodextrin elevates plasma oestradiol concentrations approximately 6 fold following vaginal administration with oestradiol benzoate (10 mg) compared with oestradiol benzoate administered without cyclodextrin.

We have also found that the cyclodextrins have indeed improved the vaginal absorption of oestradiol 17β. We have found that -cyclodextrin will approximately double the plasma oestradiol concentration when vaginally administered with oestradiol 17β (7.2 mg) compared with oestradiol 17β administered without cyclodextrin. See Figure 3. Furthermore, we have found that the cyclodextrin

hydroxypropyl β-cyclodextrin or γ-cyclodextrin elevates plasma oestradiol concentrations approximately 7 to 8 fold following vaginal administration with oestradiol (7.2 mg) compared with oestradiol 17β administered without cyclodextrin.

We have found that the molar ratio of γ-cyclodextrin to oestradiol 17β influences the vaginal absorption of oestradiol 17β. Increasing the ratio of γ-cyclodextrin to oestradiol 17β from 0.5:1 to 1:1 has been found to increase the plasma oestradiol concentration. See figures 4,5 and 6.

Further more the effect of the ratio of γ-cyclodextrin to oestradiol 17β upon the vaginal absorption of oestradiol 17β is more pronounced at higher doses (>2.5 mg). See figure 7.

We have found that vaginal administration of various amounts of oestradiol 17β (1.2, 2.5 and 7.2 mg) with various molar ratios of γ-cyclodextrin (0.5:1, 1:1 and 3:2) to oestradiol 17β has no significant effect upon the time to maximum plasma concentration (tmax) or the maximum plasma concentration (Cmax) of oestradiol. See figures 8 and 9.

We have found that the vaginal administration of a dose of oestradiol 17β greater than 2.5 mg with an amount of γ-cyclodextrin less than or equal to a molar ratio of 1:1 (γ-cyclodextrin to oestradiol 17β) results in plasma oestradiol concentrations 24 hours post administration significantly greater than those observed prior to administration. See figure 10.

We have found vaginal administration of an oestradiol 17β dose of 5 mg and γ-cyclodextrin in a molar ratio of 0.5:1 (γ-cyclodextrin to oestradiol 17β) influences follicular dynamics in a similar manner to those observed following i.m. injection of 2 mg of oestradiol benzoate. See Table 1

Table 1 tabulates a follicular dynamics and plasma oestradiol pharmacokinetics following vaginal administration of oestradiol 17β 2.5 mg or 5.0 mg and intramuscular administration of oestradiol benzoate 2 mg.

**Table 1**

| | 2.5 mg E-17 | 5.0 E-17 | 2 mg ODB |
|---|---|---|---|
| Follicular dynamics | | | |
| n | 8 | 8 | 7 |
| Day of oestrus | 22.0 ± 0.0 (n=7) | 22.0 ± 0.0 (n=8) | 22.6 ± 0.2 (n=7) |
| In oestrus by 48 h (n) | 6 | 7 | 2 |
| Follicle wave turnover (i.e. DF3) | 3 | 6 | 6 |
| No follicle wave turnover (i.e. no DF3) | 5 | 2 | 1 |
| Day DF2 emerged | 9.9 ± 0.4 [a] | 10.0 ± 0.6 [a] | 9.6 ± 0.6 [a] |
| Diameter DF2 on Day 13 (mm) | 9.9 ± 0.5 [a] | 9.3 ± 0.8 [a] | 9.6 ± 1.0 [a] |
| Growth of DF2 from Day 13-17 (mm) | 2.4 ± 0.6 [a] | 0.9 ± 0.8 [ab] | -0.6 ± 0.7 [b] |
| DF2 ovulated (n) | 5 | 2 | 0 |
| Age of ovulatory DF2 (d) | 12.0 ± 0.6 | 11.5 ± 0.5 | - |
| Diameter of ovulatory DF2 (mm) | 15.6 ± 0.7 | 17.0 ± 1.0 | - |
| Day DF3 emerged | 18.3 ± 0.5 | 15.7 ± 0.6 | 17.0 ± 0.4 |
| Interval, treatment to emergence of DF3 (d) | 5.3 ± 0.7 (4-6) | 2.7 ± 0.6 (1-5) | 4.0 ± 0.4 (3-5) |
| DF3 ovulated (n) | 1 | 6 | 5 |
| Age of ovulatory DF3 (d) | 6 | 7.0 ± 0.5 | 6.0 ± 0.5 |
| Diameter of ovulatory DF3 (mm) | 12 | 13.7 ± 0.7 | 14.2 ± 0.4 |
| **Pharmacokinetics** | | | |
| Cmax (pg/ml) | 8.8 ± 4.3 [a] | 7.1 ± 4.1 [a] | |
| Tmax (hours) | 4.9 ± 7.8 [a] | 5.9 ± 7.4 [a] | |
| AUC (pg-hr/ml) | 58.3 ± 25.8 [a] | 97.8 ± 49.0 [a] | |
| [oestradiol]pl (pg/ml) at time=0 hours | 0.17 ± 0.06 [a1] | 0.89 ± 0.13 [b1] | |
| [oestradiol]pl (pg/ml) at time=24 hours | 0.15 ± 0.04 [a1] | 1.26 ± 0.35 [b2] | |

[ab] denotes difference between groups within rows to 95% level of confidence ($p<0.05$)

[12] denotes difference between groups within columns to 95% level of confidence ($p<0.05$)

**CLAIMS:**

1. **An intra-vaginal delivery unit or composition** having an effective releasable amount of oestradiol 17β to achieve in a target mammal an efficacious effect insofar as oestrus expression is concerned.

2. **An intra-vaginal delivery unit or composition** having an effective releasable amount of oestradiol 17β to achieve in a target mammal an efficacious effect insofar as oestrus expression is concerned, and having, in addition, an agent able to enhance the absorption of the oestradiol 17β or on analogue via the vaginal route and/or the maintenance thereof in the blood of the target mammal.

3. **An intra-vaginal delivery unit or composition** having an effective amount of an active material or pro-drug thereof to achieve in a target mammal oestrus synchronisation and an effective amount of an agent to enhance the absorption by the vaginal route of the active material or pro-drug and/or the maintenance of the active material, the pro-drug or the metabolites in the blood of the target mammal.

4. **An intra-vaginal delivery unit or composition** according to claim 3 wherein the molar ratio active material or pro-drug to the agent is less than or equal to 1:1.

5. **An intra-vaginal delivery unit or composition** according to claim 3 wherein the active material or pro-drug is 2.5 mg or greater of oestradiol 17β.

6. **An intra-vaginal delivery unit or composition** according to claim 3 wherein the agent to enhance absorption is γ-cyclodextrin or hydroxypropyl β-cyclodextrin.

7. **A pharmaceutical formulation to synchronize oestrus in a target species mammal,** which comprises or includes,

(a) an active material or pro-drug thereof to achieve oestrus synchronization; and

(b) an agent (i) to enhance the absorption of the active material or pro-drug thereof and/or (ii) to ensure the appearance of the active material or its active metabolite(s) in the plasma in concentrations above those observed prior to treatment for at least 24 hours following treatment.

7. A formulation of claim 7 wherein the active material or pro-drug is oestradiol benzoate or oestradiol 17β.

9. A formulation of claim 7 or 8 wherein the agent to enhance absorption of an active material or pro-drug or to provide the longer blood serum presence of the active metabolite is a cyclodextrin.

10. A formulation of claim 9 wherein the cyclodextrin is one or more of β-cyclodextrin, γ-cyclodextrin and hydroxypropyl β-cyclodextrin.

11. A formulation of any one of claim 9 wherein the ratio of agent to enhance absorption to active material or pro-drug is less than to 3:2 (agent:active) by molecular amount, that is 3 moles of cyclodextrin to every 2 moles of active.

12. A formulation of claim 9 wherein it is in the form of an intra-vaginal dosage unit that has from 1.2 to 7.2 mg of oestradiol 17 [or an analogue equivalent amount, eg; from 10 to 30 mg if analogue is oestradiol benzoate] and from 6 to 150 mg cyclodextrin(s),

and optionally other excipients, solid or liquid,

and, optionally, if a capsule, is encased in a material such as gelatin that will release the capsule contents into vaginal fluids.

13. **A method of providing an elevated concentration of an oestrus affecting or and/or oestrus expression affecting steroid or pro-drug thereof** or **efficacious metabolites thereof** which comprises intra-vaginally administering either

(i) an effective amount of oestradiol 17β, or

(ii) an effective amount of the steroid (or analogue(s)) or pro-drug(s) thereof in conjunction (and preferably and effective amount) with an effective agent ,

to enhance the absorption by the animal of the steroid, prodrug or metabolites thereof via its vagina and/or

to achieve an appearance of the active material or active metabolite in the plasma of the animal in concentrations above those observed prior to treatment for at least 24 hours following treatment.

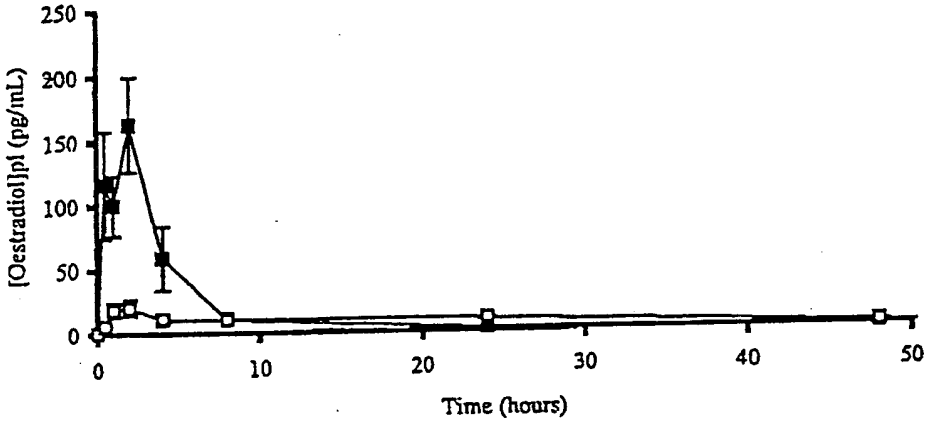
250
200
150
100
50
0
[Oestradiol]pl (pg/mL)
0
10
20
30
40
50
Time (hours)

FIGURE 1

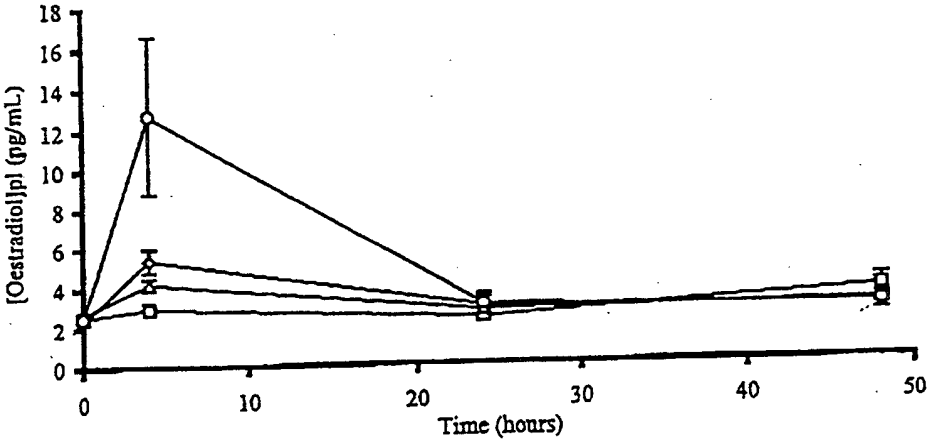
[Oestradiol]pl (pg/mL)
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
Time (hours)

FIGURE 2

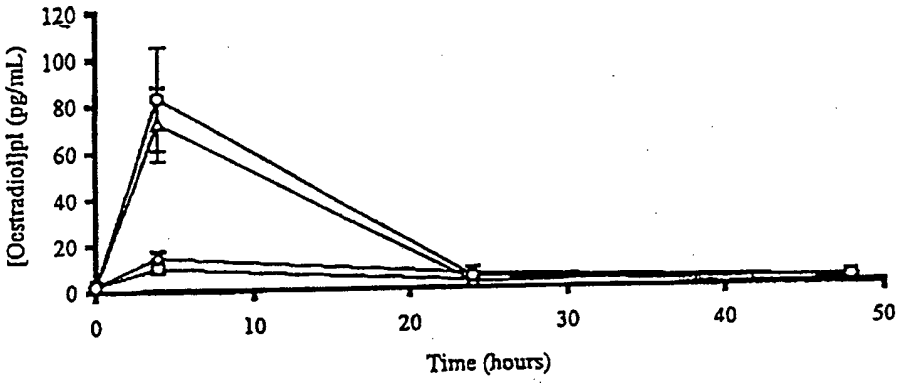
120
100
80
60
40
20
0
[Oestradiol]pl (pg/mL)
0
10
20
30
40
50
Time (hours)

FIGURE 3

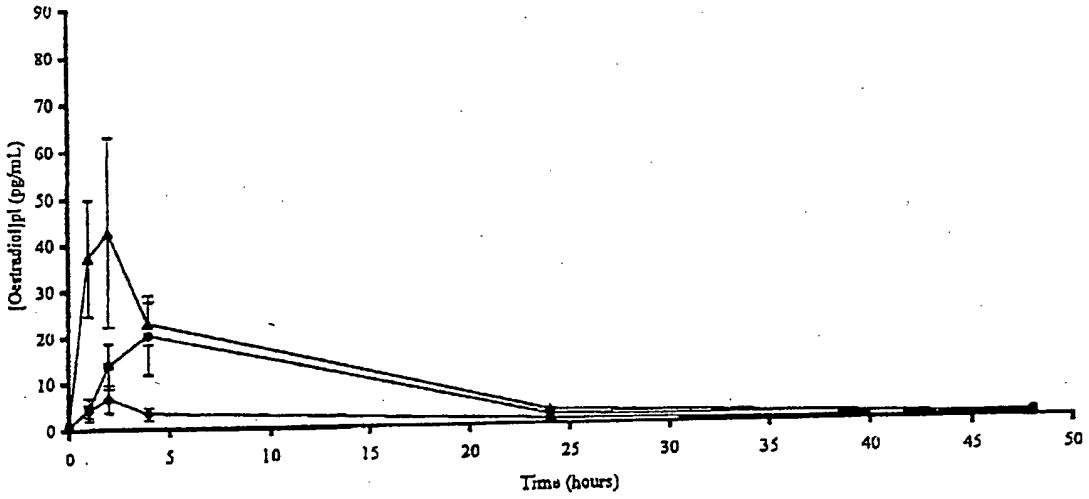
90
80
70
60
50
40
30
20
10
0
[Oestradiol]pl (pg/mL)
0
5
10
15
20
25
30
35
40
45
50
Time (hours)

FIGURE 4

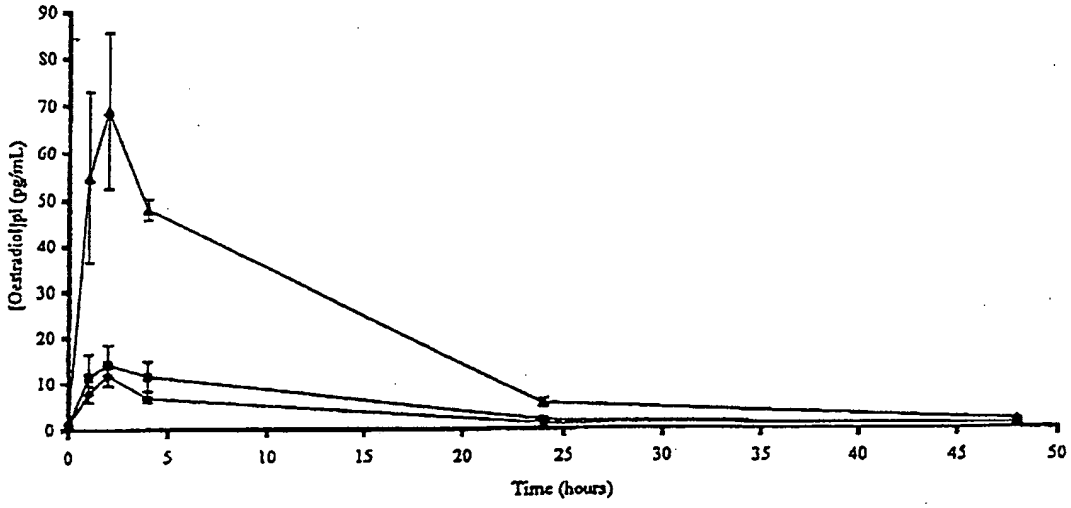
[Oestradiol]pl (pg/mL)
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
Time (hours)

FIGURE 5

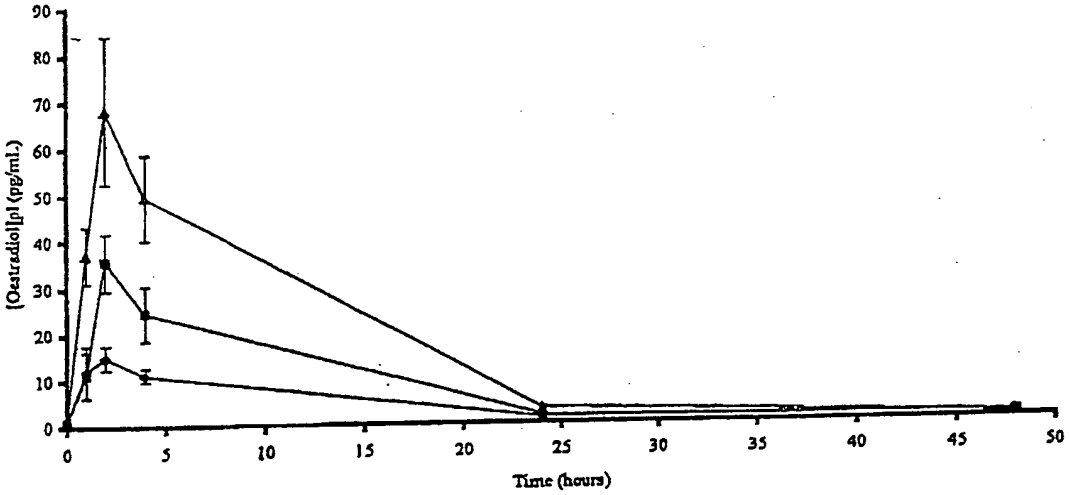
90
80
70
60
50
40
30
20
10
0
[Oestradiol]pl (pg/mL)
0
5
10
15
20
25
30
35
40
45
50
Time (hours)

FIGURE 6

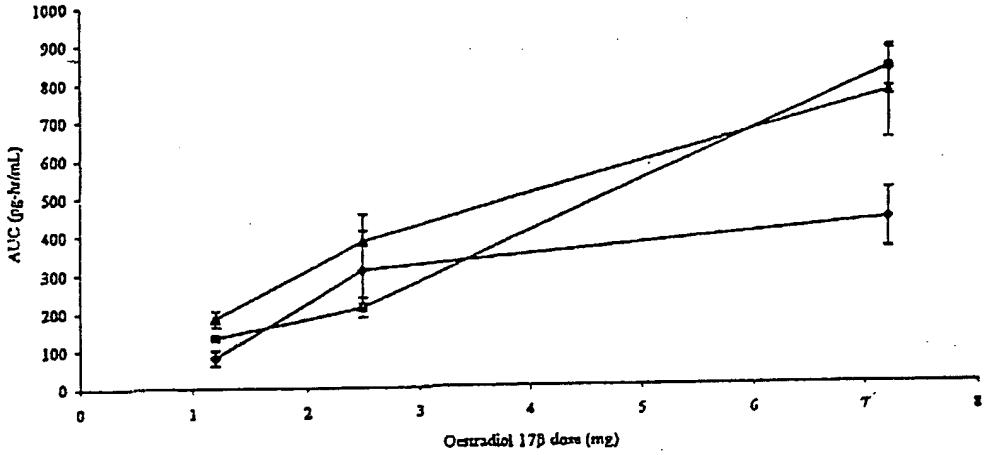
1000
900
800
700
600
500
400
300
200
100
0
AUC (pg·hr/mL)
0
1
2
3
4
5
6
7
8
Oestradiol 17β dose (mg)

FIGURE 7

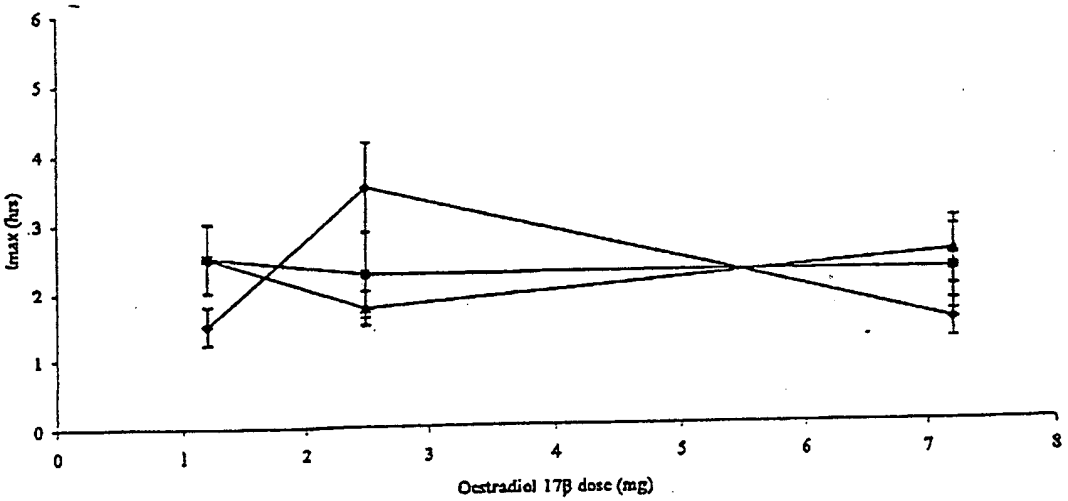


FIGURE 8

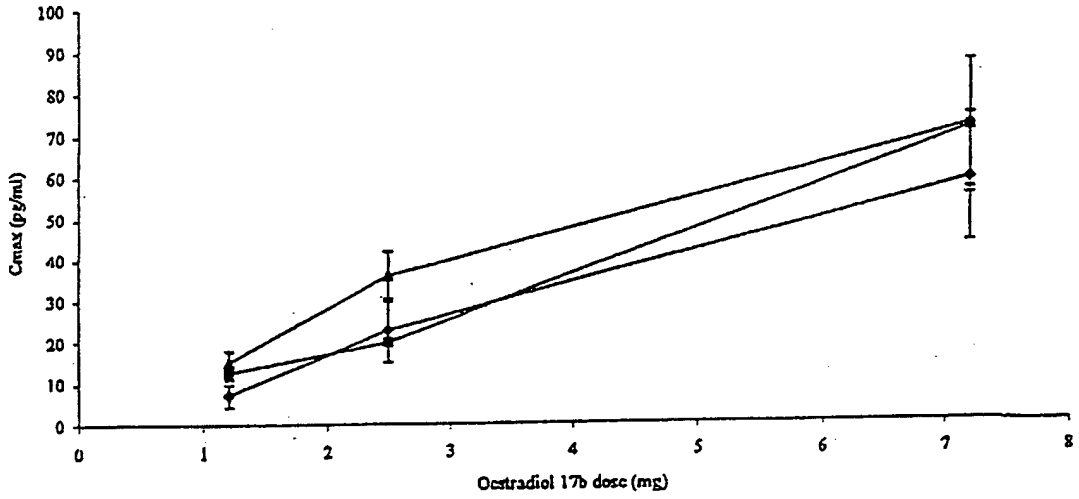
Cmax (pg/ml)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Oestradiol 17b dose (mg)

FIGURE 9

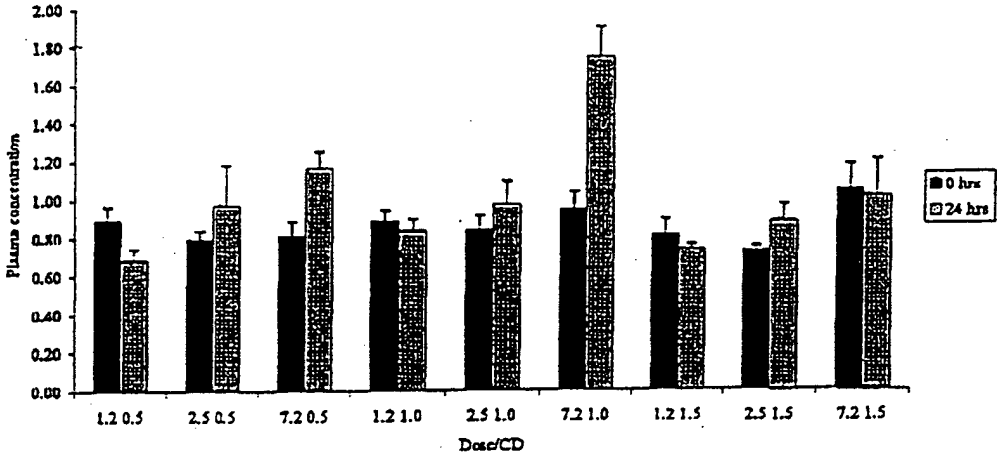
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.2 0.5
2.5 0.5
7.2 0.5
1.2 1.0
2.5 1.0
7.2 1.0
1.2 1.5
2.5 1.5
7.2 1.5
Dose/CD
0 hrs
24 hrs

FIGURE 10

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/NZ 99/00083**

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: A61K 31/565

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC: AU (AS ABOVE)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CHEMICAL ABSTRACTS

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: oestradiol or estradiol and cyclodextrin

**C. *DOCUMENTS CONSIDERED TO BE RELEVANT***

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | AU 74111/94 A (ADIR ET COMPAGNIE) 6 April 1995<br>See Examples 1 & 2 | 1-12 |
| X | US 4877774 A (PITHA et al.) 31 October 1989<br>See column 2 lines 56-65, column 3 lines 60 - column 4 line 2, claims 1-2, 4-5 | 1-12 |
| X | US 4642305 A (JOHANSSON ET AL.) 10 February 1987<br>See column 2 lines 10-25, 47-51, claim 1. | 1-12 |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 September 1999 | -8 OCT 1999 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No.: (02) 6285 3929 | Authorized officer<br><br>**TAMARA NIZNIK**<br>Telephone No.: (02) 6283 2422 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ 99/00083

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 349091 A1 (HERMENS, Walter Adrianus et al.) 3 January 1990, See page 2 lines 32-46, claims 1-5. | 1-12 |
| P,X | DE 19734538 C1 (JENAPHARM GmbH) 24 December 1998 | 1-13 |
| X | Pharmazie Vol 51, Number 1, 1996 pages 39-42, (FRIDRIKSDOTTIR et al.) 'Design and in vivo testing of 17β- estradiol-HPBCD sublingual tablets' see abstract. | 1-12 |
| X | European Journal of Pharmaceutics and Biopharmaceutics Vol 42 (5), 1996, pages 320-324, (KUBLIK et al.) 'Nasal absorption of 17-β- Estradiol from different cyclodextrin inclusion formulations in sheep'. See summary and discussion. | 1-12 |
| X | Chemical Abstracts, 1997: 194543 CAPLUS BREWSTER M, E (et al.) 'Intravenous and buccal 2-hydroxypropyl - beta - cyclodextin formulations of E2 - CDS - Phase I clinical trials.' Abstract. | 1-12 |

# INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
**PCT/NZ 99/00083**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
| AU | 74111/94 | CA | 2132511 | EP | 649653 | FR | 2710268 |
| | | JP | 7247223 | ZA | 9407401 | | |
| US | 4642305 | EP | 160071 | WO | 8501875 | | |
| EP | 349091 | NL | 8801670 | US | 5089482 | | |
| DE | 19734538 | EP | 894495 | | | | |

END OF ANNEX